REMARKS

Rejection under §101

The Claims have been amended to recite an "isolated transformed mammalian cell."

Rejections under §112

1) Hybridization claims are expressly approved in Patent Office Guidelines, and consistently, have been routinely granted by the U.S. Patent Office. The current rejection set forth in the Office action is clearly contrary to Patent Office policy and statutory authority.

The Written Description Guidelines

The Patent Office's *Written Description Guidelines* establish that hybridization claims to §112, first paragraph. For instance, Example 9 of the *Guidelines* provides an example of a claim that covers sequences that hybridize to a recited sequence, and which encode proteins with a particular activity. ("An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO:1, wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.") The elements set forth in the *Guidelines* as being adequate to fulfill the written description requirements included: (1) the protein's dopaminergic activity; (2) "a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus"; and (3) hybridization conditions that "yield structurally similar DNAs."

The pending claims meet these criteria. They include a functional activity("upon activation by histamine, leads to the accumulation of cAMP"), a disclosed species that meets the criteria, and hybridization conditions. Taken together, it is evident that the specification clearly

provides the information set forth by the U.S. Patent Office as needed to meet the statutory requirements for a hybridization claim.

The Patent Office has consistently granted hybridization claims

The Patent Office has issued hundreds of claims having the hybridization claim format. For example, a search of recently published patents included the following seven patents, each which recite hybridizations claims, e.g., U.S. Pat. Nos. 6,790,629; 6,787,645; 6,759,225; 6,734,284; 6,586,179; 6,657,107; and 6,635,446 (see attaché). The examiner, himself, has issued patents having hybridization claims. See, e.g., U.S. Pat. No. 6,699,660 and 6,413,741 (see attached).

2) As explained in the specification, the biological activity of the H2 receptor is well known in the art. See, e.g., Specification, Page 1, line 25-Page 4, line 16. See, also U.S. Pat. No. 5,885,824, Column 1, lines 20-37; Column 2, lines 60-65; Column 3, line 65-Column 4, line 6; Column 4, lines 54-60. The Examiner has provided no evidence to support the allegations in the Office action about lack of written description and indefiniteness. Thus, the rejection cannot be maintained. However, to expedite prosecution, the claim to "human H2R polynucleotide" has been amended to recite that the claimed polypeptide "upon activation by histamine, leads to the accumulation of cAMP." This unnecessary amendment is supported in the specification, e.g., on Page 3, lines 10-17.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Date: October 22, 2004

United States Patent **6,790,629**
Julius , et al. **September 14, 2004**

Nucleic acid sequences encoding capsaicin receptor and capsaicin receptor-related polypeptides and uses thereof

Abstract

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human disease and painful syndromes.

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Assignee: **The Regents of the University of California** (Oakland, CA)
Appl. No.: **978303**
Filed: **October 15, 2001**

Current U.S. Class: **435/7.1**
Intern'l Class: G01N 033/53; G01N 033/567
Field of Search: 435/7.1,7.21,69.1 436/501 536/23.5

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GenBank Accession No. N24224.
GenBank Accession No. W92895.
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Primary Examiner: Ulm; John D.
Attorney, Agent or Firm: Borden; Paula A., Francis; Carol L. Bozicevic, Field & Francis, LLP

Parent Case Text

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 09/235,451, filed Jan. 22, 1999, now U.S. Pat. No. 6,335,180, which is a continuation-in-part of: 1) U.S. Provisional patent application Ser. No. 60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997, now abandoned; and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, which PCT International application was published in English on Jul. 29, 1999 as WO 9937675, each of which applications are incorporated herein by reference.

Claims

What is claimed is:

1. A method of screening for an agent that modulates capsaicin receptor function, the method comprising:

a) combining a candidate agent with a eukaryotic cell comprising a recombinant nucleic acid comprising a nucleotide sequence that encodes a biologically active capsaicin receptor polypeptide, which nucleotide sequence is operably linked to a promoter, wherein said capsaicin receptor is encoded by a polynucleotide that hybridizes under stringent ***hybridization conditions*** to the complement of a polynucleotide having the sequence set forth in SEQ ID) NO: 1, and wherein the capsaicin receptor polypeptide is expressed on the cell surface; and

b) determining the effect of said agent on capsaicin receptor function.

2. The method of claim 1, wherein said determining is by measuring capsaicin receptor-mediated increase in intracellular concentration of a cation.

3. The method of claim 2, wherein the cation is selected from the group consisting of calcium, magnesium, potassium, cesium, and sodium.

4. The method of claim 2, wherein the cation is calcium.

5. The method of claim 1, wherein said determining is by measuring a capsaicin receptor-mediated electrophysiological response.

6. The method of claim 5, wherein the electrophysiological response is an inward cation-specific current.

7. The method of claim 5, wherein the response is measured using a fluorescent voltage-sensitive dye.

8. The method of claim 1, wherein said determining is by measuring blocking the activity of a capsaicin

receptor antagonist.

9. The method of claim 8, wherein the capsaicin receptor antagonist is selected from the group consisting of capsazepine and ruthenium red.

10. The method of claim 1, wherein said determining is by measuring blocking the activity of a capsaicin receptor agonist.

11. The method of claim 10, wherein the capsaicin receptor agonist is selected from the group consisting of resiniferatoxin and capsaicin.

12. The method of claim 1, wherein said determining is by measuring capsaicin receptor-mediated apoptosis.

13. The method of claim 1, wherein said cell further comprises a reporter gene operably linked to a calcium inducible promoter, and wherein said determining is by measuring calcium-induced expression of the reporter gene.

14. The method of claim 1, wherein the cell is selected the group consisting of an amphibian oocyte, a mammalian cell line, and a cultured neuron.

15. The method of claim 1, wherein the capsaicia receptor is a mammalian capsaicin receptor.

Description

FIELD OF THE INVENTION

The present invention relates to nucleic acid and amino acid sequences encoding a receptor for vanilloid compound and polypeptides related to such vanilloid compound receptors, and to the use of these sequences in the diagnosis, study, and treatment of disease.

BACKGROUND OF THE INVENTION

Pain is initiated when the peripheral terminals of a particular group of sensory neurons, called nociceptors, are activated by noxious chemical, mechanical, or thermal stimuli. These neurons, whose cell bodies are located in various sensory ganglia, transmit information regarding tissue damage to pain processing centers in the spinal cord and brain (Fields Pain (McGraw-Hill, New York, 1987)). Nociceptors are characterized, in part, by their sensitivity to capsaicin, a natural product of capsicum peppers that is the active ingredient of many "hot" and spicy foods. In mammals, exposure of nociceptor terminals to capsaicin leads initially to the perception of pain and the local release of neurotransmitters. With prolonged exposure, these terminals become insensitive to capsaicin, as well as to other noxious stimuli (Szolcsanyi in Capsaicin in the Study of Pain (ed. Wood) pgs. 255-272 (Academic Press, London, 1993)). This latter phenomenon of nociceptor desensitization underlies the seemingly paradoxical use of capsaicin as an analgesic agent in the treatment of painful disorders ranging from viral and diabetic neuropathies to rheumatoid arthritis (Campbell in Capsaicin and the Study of Pain (ed. Wood) pgs. 255-272 (Academic Press, London, 1993); Szallasi et al. 1996 Pain 68:195-208). While some of this decreased sensitivity to noxious stimuli may reflect reversible changes in the nociceptor, such as depletion of inflammatory mediators, the long-term loss of responsiveness can be explained by death of the nociceptor or destruction of its peripheral terminals following capsaicin exposure (Jancso et

United States Patent
Rollins , et al.

6,787,645
September 7, 2004

DNA encoding human JE cytokine

Abstract

A novel human cytokine, JE factor, and processes for producing it are disclosed. JE may be used in pharmaceutical preparations for stimulating and/or enhancing immune responsiveness and in wound healing and related tissue repair. containing the factor.

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Appl. No.: **437306**
Filed: **May 9, 1995**

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Intern'l Class: C12N 005/10; C12N 015/19; C12N 015/63; C12N 014/52
Field of Search: 435/69.5,252.3,240.2,320.1,172.3,471,325 536/23.5,24.3,24.31 530/324 935/11,22,66,27

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Primary Examiner: Mertz; Prema

Attorney, Agent or Firm: Hamilton, Brook, Smith & Reynolds, P.C.

Parent Case Text

This application is a division of co-pending application Ser. No. 08/228,931 filed Apr. 13, 1994 now abandoned which is a Continuation of 08/003,136 filed Jan. 12, 1993 now abandoned which is a CIP of Ser. No. 07/701,515, filed May 16, 1991, now U.S. Pat. No. 5,179,078, which is a CIP of Ser. No. 07/351,008, filed May 12, 1989, now U.S. Pat. No. 5,212,073.

Claims

What is claimed is:

1. An isolated or recombinant DNA as set forth in SEQ ID NO: 1.

2. An isolated or recombinant DNA as set forth from nucleotide 73 to nucleotide 772 of SEQ ID NO: 1.

3. An isolated or recombinant DNA as set forth from nucleotide 160 to nucleotide 369 of SEQ ID NO: 1.

4. An isolated or recombinant DNA as set forth from nucleotide 144 to nucleotide 369 of SEQ ID NO: 1.

5. An isolated or recombinant DNA as set forth from nucleotide 73 to nucleotide 369.

6. An isolated or recombinant DNA encoding a protein as set forth from amino acid 30 to amino acid 99 of SEQ ID NO: 2.

7. An isolated or recombinant DNA encoding a protein as set forth from amino acid 24 to amino acid 99 of SEQ ID NO: 2.

8. An isolated or recombinant DNA encoding a protein as set forth in SEQ ID NO: 2.

9. An isolated or recombinant DNA which hybridizes to the complement of the sequence of SEQ ID NO: 1, wherein said isolated or recombinant DNA hybridizes under ***hybridization conditions*** of 4.times.SSC at 65.degree. C., followed by a washing in 0.1 XSSC at 65.degree. C. for an hour, or under ***hybridization conditions*** of 50% formamide, 4.times.SSC at 42.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, and wherein said isolated or recombinant DNA encodes a protein which suppresses tumor formation.

10. An isolated or recombinant DNA which hybridizes to the complement of DNA as set forth in nucleotide 73 to nucleotide 772 of SEQ ID NO: 1, wherein said isolated or recombinant DNA hybridizes under hybridization conditions of 4.times.SSC at 65.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, or under conditions of 50% formamide, 4.times.SSC at 42.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, and wherein said isolated or recombinant DNA encodes a protein which suppresses tumor formation.

11. An isolated or recombinant DNA which hybridizes to the complement of DNA as set forth in nucleotide 160 to nucleotide 369 of SEQ ID NO: 1, wherein said isolated or recombinant DNA hybridizes under conditions of 4.times.SSC at 65.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, or under conditions of 50% formamide, 4.times.SSC at 42.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, and wherein said isolated or recombinant DNA encodes a protein which suppresses tumor formation.

12. An isolated or recombinant DNA which hybridizes to the complement of DNA as set forth in nucleotide 144 to nucleotide 369 of SEQ ID NO: 1, wherein said isolated or recombinant DNA hybridizes under conditions of 4.times.SSC at 65.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, or under conditions of 50% formamide, 4.times.SSC at 42.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, and wherein said isolated or recombinant DNA encodes a protein which suppresses tumor formation.

13. An isolated or recombinant DNA which hybridizes to the complement of DNA as set forth in nucleotide 73 to nucleotide 369 of SEQ ID NO: 1, wherein said isolated or recombinant DNA hybridizes under conditions of 4.times.SSC at 65.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, or under conditions of 50% formamide, 4.times.SSC at 42.degree. C., followed by a washing in 0.1.times.SSC at 65.degree. C. for an hour, and wherein said isolated or recombinant DNA encodes a protein which suppresses tumor formation.

Description

The present invention relates to a novel cytokine that is important in host defense and immunity against infection and for the processes for obtaining the purified factor by recombinant genetic engineering techniques.

BACKGROUND OF THE INVENTION

A family of regulatory proteins that deliver signals between many different types of cells in the body has been identified. These regulatory molecules are known as cytokines. Many of the cytokines have been found to control the growth and development and biological activities of cells in the hematopoietic and immune systems. Cytokines have also been identified which are produced by other cell types including fibroblasts and endothelial cells which transmit signals between these cells and a variety of responsive target cells. This family of cytokines is clearly important for maintaining homeostasis and for coordinating the physiological responses to a variety of insults including wounding and infection as well as regulating the immune response [See, for example G. Wong & S. Clark, Immunology Today, 9 (5):139 (1988)]. The family of cytokines includes the interleukins, the hematopoietic colony-stimulating factors, the interferons, and the tumor necrosis factors among others. In addition, two subfamilies within the larger cytokine family have emerged that share evolutionary relatedness at the nucleotide level. Members of one of these families share sequence similarity with a cytokine known as macrophage inflammatory protein 1 (MIP-1) [Davatelis, G. et al J. Exp. Med., 167:1939-1944 (1988)], while members of the other family share sequence similarity with a second macrophage inflammatory protein, MIP-2 [Wolpe, S. D. et al, Proc. Nat'l Acad. Sci. USA, 86:612-616 (1988)]. MIP-1 and MIP-2 are cytokines produced by activated macrophages that induce local inflammatory responses when injected subcutaneously in mice. Other polypeptides have been identified through molecular biological approaches which are clearly related to either MIP-1 or MIP-2 but for which biological activities have not yet been identified. Although the function of these molecules is not known, they, like other members of the cytokine family, are likely to be important in various aspects of regulating homeostasis or

coordinating physiological responses to wounding, injury, or infection or in the regulation of the immune system.

One member of the MIP-1 subfamily may be the murine JE [Rollins et al, Proc. Nat.'l. Acad. Sci. USA 85:3738-3752 (1988)] and its human homolgue disclosed herein.

BRIEF SUMMARY OF THE INVENTION

In one aspect the present invention provides, substantially free from co-produced polypeptides, a novel human cytokine herein termed JE which is elicited in response to platelet-derived growth factor (PDGF). JE may be characterized by containing the predicted amino acid sequence from at least amino acid #30 to #99 as set forth in Table I. This novel factor when expressed in COS cells displays considerable size heterogeneity with three predominant species present with estimated sizes of approximately 15,500, 15,000, and 13,000 as determined by SDS-PAGE. Additional microheterogeneous species are present with molecular weights from 16,000-18,000 daltons.

In one aspect, the invention provides JE factor produced by culturing a cell transformed with the DNA sequence comprising the sequence of Table I from at least nucleotide #73 to #772 and recovering and purifying from the culture medium a protein comprising the amino acid sequence from amino acid #30 to #99 of Table I.

Another aspect of the invention includes DNA sequences coding on expression for a human JE polypeptide. One such DNA sequence is the same or substantially the same as the approximately 772 nucleotide sequence which appears below in Table I.

Also provided by the present invention are vectors containing a DNA sequence encoding JE in operative association with an expression control sequence. Host cells transformed with such vectors for use in producing recombinant JE are also provided by the present invention.

The vectors and transformed cells of the invention are employed in another aspect, a novel process for producing recombinant human JE polypeptide. In this process a cell line transformed with a DNA sequence encoding JE polypeptide in operative association with an expression control sequence therefor is cultured. This claimed process may employ a number of known cells as host cells for expression of the polypeptide. Presently preferred cell lines are mammalian cell lines, and bacterial cells.

Another aspect of this invention provides pharmaceutical compositions comprising a therapeutically effective amount of JE in a pharmaceutically acceptable vehicle. Because JE expression is activated by PDGF, a growth factor released by platelets at the site of a wound, JE protein is likely to be useful directly for treating wounds. JE is also likely to have other cytokine properties including the ability to enhance host defense or to stimulate the hematopoietic or immune systems. Therefore, the pharmaceutical compositions of the invention may be useful in the treatment of cancer or in potentiating the efficacy of vaccines. Generally, it is contemplated that compositions of the invention may be useful for the treatment of disease states which involve immune system deficiencies.

A further aspect of the invention, therefore, is a method for treating tissue injuries or accelerating wound healing by administering to a patient a therapeutically effective amount of JE in a suitable pharmaceutical carrier. Further included are methods for treating cancer, diseases characterized by a deficiency in the number or level of activity of hematopoietic cells, or potentiating the efficacy of vaccines by administering to a patient a therapeutically effective amount of JE in a suitable pharmaceutical carrier. These therapeutic methods may include administering simultaneously or sequentially with JE polypeptides an effective amount of at least one other cytokine, hematopoietin,

United States Patent **6,759,225**
Udagawa , et al. **July 6, 2004**

Lysophospholipase

Abstract

The inventors have isolated lysophospholipases from Aspergillus (A. niger and A. oryzae) having molecular masses of about 68 kDa and amino acid sequences of 600-604 amino acid residues. The novel lysophospholipases have only a limited homology to known amino acid sequences. The inventors also isolated genes encoding the novel enzymes and cloned them into E. coli strains.

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Assignee: **Novozymes A/S** (Bagsvaerd, DK)

Appl. No.: **309437**

Filed: **December 4, 2002**

Foreign Application Priority Data

Oct 14, 1999[DK] 1999 01473

Current U.S. Class: **435/195; 435/196; 435/266; 435/267**
Intern'l Class: C12N 009/20
Field of Search: 435/198,195,196,197,266,267 536/23.2

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6146869	Nov., 2000	Harris et al.	435/198.

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98/31790	Jul., 1998	WO.

Other References

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Mustranata el al, Process Biochemistry, pp. 393-401 (1995).

Primary Examiner: Nashed; Nashaat T.
Attorney, Agent or Firm: Garbell; Jason, Lambiris; Elias

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application divisional of U.S. application Ser. No. 09/687,538, filed Oct. 13, 2000, now U.S. Pat. No. 6,514,759, which is as continuation of U.S. application Ser. No. 09/678.513, filed on Oct. 3, 2000, now abandoned, and claims priority of Danish application no. PA 1999 01473, filed Oct. 14, 1999, and U.S. provisional application No. 60/160,572 filed. Oct. 20, 1999, the contents of which are fully incorporated herein by reference.

Claims

What is claimed is:

1. An isolated lysophospholipase, comprising:

a) a polypeptide encoded by a lyphospholipase encoding part of the DNA sequence cloned into a plasmid present in Escherichia coli deposit number DSM 13083;

b) a polypeptide having an amino acid sequence of amino acids 1-603 in SEQ ID NO: 8;

c) an analogue of the polypeptide defined in (a) or (b) which has at least 95% sequence homology with said polypeptide; or

d) a polypeptide which is encoded by a nucleic acid sequence which hybridizes with a complementary strand of the nucleic acid sequence shown as nucleotides 193-2001 of SEQ ID NO:7 under hybrdizaion conditions comprising prehybridizing in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approx. 45.degree. C., followed by washing in 2.times.SSC, 0.5% SDS for 30 minutes at a temperature of at least 65.degree. C.

2. The lysophospholipase of claim 1 which is native to a strain of Aspergillus.

3. The lysophospholipase of claim 1, which is native to a strain of A. oryzae.

4. The lysophospholipase of claim 1, comprising a deletion of 25-35 amino acids at the C-terminal end.

5. The lysophospholipase of claim 1, comprising a polypeptide that is encoded by a nucleic acid sequence which hybridizes with a complementary strand of the nucleic acid sequence shown as nucleotides 193-2001 of SEQ ID NO:7 under ***hybridization conditions*** comprising prehybridizing in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approx. 45.degree. C., followed by washing in 2.times.SSC, 0.5% SDS for 30 minutes at a temperature of at least 65.degree. C.

6. The lysophospholipase of claim 1, comprising a polypeptide that is encoded by a nucleic acid sequence which hybridizes with a complementary strand of the nucleic acid sequence shown as nucleotides 193-2001 of SEQ ID NO:7 under ***hybridization conditions*** comprising prehybridizing in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approx. 45.degree. C., followed by washing in 2.times.SSC, 0.5% SDS for 30 minutes at a temperature of at least 70.degree. C.

7. The lysophospholipase of claim 1, comprising a polypeptide that is encoded by a nucleic acid sequence which hybridizes with a complementary strand of the nucleic acid sequence shown as nucleotides 193-2001 of SEQ ID NO:7 under ***hybridization conditions*** comprising prehybridizing in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approx. 45.degree. C., followed by washing in 2.times.SSC, 0.5% SDS for 30 minutes at a temperature of at least 75.degree. C.

8. The lysophospholipase of claim 1, comprising a polypeptide which has at least 95% homology to the polypeptide encoded by a lysophospholipase encoding part of the DNA sequence cloned into a plasmid present in Escherichia coli deposit number DSM 13083 or to the polypeptide having an amino acid sequence of amino acids 1-603 in SEQ ID NO: 8.

9. The lysophospholipase of claim 1, comprising a polypeptide which has at least 98% homology to the polypeptide encoded by a lysophospholipase encoding part of the DNA sequence cloned into a plasmid present in Escherichia coli deposit number DSM 13083 or to the polypeptide having an amino acid sequence of amino acids 1-603 in SEQ ID NO: 8.

10. The lysophospholipase of claim 1, comprising a polypeptide encoded by a lysophospholipase encoding part of the DNA sequence cloned into a plasmid present in Escherichia coli deposit number DSM 13083.

11. The lysophospholipase of claim 1, comprising a polypeptide having an amino acid sequence comprising amino acids 1-603 in SEQ ID NO: 8.

12. A process for hydrolyzing fatty acyl groups in a phospholipid or lysophospholipid, comprising treating the phospholipid or lysophospholipid with the lysophospholipase of claim 1.

13. A process for improving the filterability of an aqueous solution or slurry of carbohydrate origin which contains phospholipid which process comprises treating the solution or slurry with the lysophospholipase of claim 1.

14. The process of claim 28, wherein the solution or slurry contains a starch hydrolysate.

15. The process of claim 28, wherein the solution or slurry contains a wheat starch hydrolysate.

Description

FIELD OF THE INVENTION

The present invention relates to lysophospholipases (LPL), methods of using and producing them, as well as nucleic acid sequences encoding them.

BACKGROUND OF THE INVENTION

Lysophospholipases (EC 3.1.1.5) are enzymes that can hydrolyze 2-lysophospholids to release fatty acid. They are known to be useful, e.g., for improving the filterability of an aqueous solution containing a starch hydrolysate, particularly a wheat starch hydrolysate (EP 219.269).

N. Masuda et al., Eur. J. Biochem., 202, 783-787 (1991) describe an LPL from Penicillium notatum as a glycoprotein having a molecular mass of 95 kDa and a published amino acid sequence of 603 amino acid residues. WO 98/31790 and EP 808,903 describe LPL from Aspergillus foetidus and Aspergillus niger, each having a molecular mass of 36 kDa and an amino acid sequence of 270 amino acids.

JP-A 10-155493 describes a phospholipase A1 from Aspergillus oryzae. The mature protein has 269 amino acids.

SUMMARY OF THE INVENTION

The Inventors have isolated lysophospholipases from Aspergillus (A. niger and A. oryzae) having molecular masses of about 68 kDa and amino acid sequences of 600-604 amino acid residues. The novel lysophospholipases have only a limited homology to known amino acid sequences. The inventors also isolated genes encoding the novel enzymes and cloned them into E. coil strains.

Accordingly, the invention provides a lysophospholipase which may be a polypeptide having an amino acid sequence as the mature peptide shown in one of the following or which can be obtained therefrom by substitution, deletion, and/or insertion of one or more amino acids, particularly by deletion of 25-35 amino acids at the C-terminal:

SEQ ID NO: 2 (hereinafter denoted A. niger LLPL-1),

SEQ ID NO: 4 (hereinafter denoted A. niger LLPL-2),

SEQ ID NO: 6 (hereinafter denoted A. oryzae LLPL-1), or

SEQ ID NO: 8 (hereinafter denoted A. oryzae LLPL-2).

Further, the lysophospholipase of the invention may be a polypeptide encoded by the lysophospholipase encoding part of the DNA sequence cloned into a plasmid present in Escherichia coli deposit number DSM 13003, DSM 13004, DSM 13082 or DSM 13083.

United States Patent **6,734,284**
Johansen , et al. **May 11, 2004**

Neublastin neurotrophic factors

Abstract

The invention relates to neublastin neurotrophic factor polypeptides, nucleic acids encoding neublastin polypeptides, and antibodies that bind specifically to neublastin polypeptides, as well as methods of making and methods of using the same.

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Field of Search: 530/300,350 514/2-12

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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional application of U.S. patent application Ser. No. 09/347,613 (now U.S. Pat. No. 6,593,133), filed Jul. 2, 1999, which claims the benefit of U.S. Serial No. 60/092,229, filed Jul. 9, 1998; U.S. Serial No. 60/097,774, filed Aug. 25, 1998, and U.S. Serial No. 60/103,908, filed Oct. 13, 1998 each hereby incorporated by reference in their entirety. This application also claims benefit of Danish patent application 1998 00904, filed Jul. 6, 1998; Danish patent application 1998 01048, filed Aug. 19, 1998 and Danish patent application 1998 01265, filed Oct. 6, 1998.

Claims

We claim:

1. An isolated neublastin polypeptide with neurotrophic activity comprising the following:

(a) seven conserved cysteine residues at positions 8, 35, 39, 72, 73, 101, and 103 when numbered in accordance with SEQ ID NO. 2;

(b) amino acid residues as follows:

C at position 8, L at position 10, V at position 17, L at position 20, G at position 21, L at position 22, G at position 23, E at position 28, F at position 32, R at position 33, F at position 34, C at position 35, G at position 37, C at position 39, C at position 72, C at position 73, R at position 74, P at position 75, F at position 83, D at position 85, S at position 97, A at position 98, C at position 101 and C at position 103, each when numbered in accordance with SEQ ID NO. 2;

(c) an LGLG repeat, an FRFC motif, a QPCCRP motif, and a SATACGC motif; and

(d) an amino acid sequence comprising at least 90% sequence identity to AA.sub.1 -AA.sub.105 of SEQ ID NO. 2.

2. The polypeptide of claim 1, wherein said polypeptide is coded for by a nucleic acid selected from the group consisting of:

a) nucleotides 405-719 of SEQ ID NO. 1; and

b) a nucleotide sequence that hybridises specifically to a complement sequence of nucleotides 405-719 of SEQ ID NO. 1 under high stringency solution ***hybridization conditions where the hybridization conditions*** comprise pre-soaking of a filter containing the sequence of nucleotides in 5.times.Sodium chloride/Sodium citrate (SSC) for 10 minutes, pre-hybridization of the filter in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml of denatured sonicated salmon sperm DNA, hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed .sup.32 P-dCTP-labeled (specific activity>1.times.10.sup.9 cpm/.mu.g) probe for 12 hours at approximately 45.degree. C. and washing the filter for thirty minutes in 0.1.times.SSC, 0.5% SDS at a temperature of at least 70.degree. C.;

wherein the nucleotide sequence codes on-expression for a neublastin polypeptide comprising all of the following characteristics:

(i) seven conserved cysteine residues at positions 8, 35, 39, 72, 73, 101, and 103 when numbered in accordance with SEQ ID NO. 2;

(ii) amino acid residues as follows:

C at position 8, L at position 10, V at position 17, L at position 20, G at position 21, L at position 22, G at position 23, E at position 28, F at position 32, R at position 33, F at position 34, C at position 35, G at position 37, C at position 39, C at position 72, C at position 73, R at position 74, P at position 75, F at position 83, D at position 85, S at position 97, A at position 98, C at position 101 and C at position 103, each when numbered in accordance with SEQ ID NO. 2;

(iii) an LGLG repeat, an FRFC motif, a QPCCRP motif, and a SATACGC motif; and

(iv) an amino acid sequence having at least 90% sequence identity with AA.sub.1 -AA105 of SEQ ID NO. 2.

3. The neublastin polypeptide of claim 1, wherein the polypeptide has a C-terminal amino acid sequence as set forth in AA.sub.72 -AA.sub.105 of SEQ. ID. NO. 2.

4. The neublastin polypeptide of claim 1 wherein the polypeptide has a C-terminal amino acid sequence as set forth in AA.sub.41 -AA.sub.105 of SEQ. ID. NO. 2.

5. The neublastin polypeptide of claim 1 comprising conservative amino acid substitutions, wherein the conservative amino acid substitutions represent less than 10% of the total number of residues in the polypeptide.

6. The neublastin polypeptide of claim 5 wherein the conservative amino acid substitutions represent less than 2% of the polypeptide.

7. The neublastin polypeptide of claim 5 wherein the conservative amino acid substitutions represent a single amino acid substitution in the mature sequence, wherein both the substituted and replacement amino acids are non-cyclic.

8. The polypeptide according to any one of claims 1-7 wherein said polypeptide is glycosylated.

9. A composition comprising the polypeptide according to any one of claims 1-8 and a pharmaceutically acceptable carrier.

Description

FIELD OF THE INVENTION

The invention relates to neurotrophic factor polypeptides, nucleic acids encoding neurotrophic factor polypeptides, and antibodies that bind specifically to neurotrophic factors.

BACKGROUND

Neurotrophic factors are naturally-occurring proteins which promote survival, maintain phenotypic differentiation, prevent degeneration, and enhance the activity of neuronal cells and tissues. Neurotrophic factors are isolated from neural tissue and from non-neural tissue that is innervated by the nervous system, and have been classified into functionally and structurally related groups, also referred to as families, superfamilies, or subfamilies. Among the neurotrophic factor superfamilies are the fibroblast growth factor, neurotrophin, and transforming growth factor-.beta. (TGF-.beta.) superfamilies. Individual species of neurotrophic factors are distinguished by their physical structure, their interaction with their composite receptors, and their affects on various types of nerve cells. Classified within the TGF-.beta. superfamily (Massague, et al,. 1994, Trends in Cell Biology, 4:172-178) are the glial cell line-derived neurotrophic factor ligands ("GDNF"; WO 93/06116, incorporated herein by reference), which include GDNF, persephin ("PSP"; Milbrandt et al., 1998, Neuron 20:245-253, incorporated herein by reference) and neurturin ("NTN"; WO 97/08196, incorporated herein by reference). The ligands of the GDNF subfamily have in common their ability to induce signalling through the RET receptor

United States Patent **6,586,179**
Jegla , et al. **July 1, 2003**

Human Eag2

Abstract

The invention provides isolated nucleic acid and amino acid sequences of Eag2, antibodies to Eag2, methods of detecting Eag2, and methods of screening for modulators of Eag2 potassium channels using biologically active Eag2. The invention further provides, in a computer system, a method of screening for mutations of human Eag2 genes as well as a method for identifying a three-dimensional structure of Eag2 polypeptide monomers.

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Field of Search: 536/23.1 435/7.1,325,320.1,252.3 530/350 436/6

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Parent Case Text

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 60/143,467, filed Jul. 13, 1999, herein incorporated by reference in its entirety.

Claims

What is claimed is:

1. An isolated nucleic acid encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and wherein said nucleic acid specifically hybridizes under stringent conditions to SEQ ID NO:1, wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

2. An isolated nucleic acid encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and

(ii) comprises an amino acid sequence that has greater than 85% amino acid identity to the amino acid sequence of SEQ ID NO:2.

3. The isolated nucleic acid of claim 1, wherein the polypeptide specifically binds to polyclonal antibodies generated against SEQ ID NO:2.

4. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes human Eag2.

5. The isolated acid of claim 1, wherein the nucleic acid encodes an amino acid sequence of SEQ ID NO:2.

6. The isolated nucleic acid sequence of claim 1, wherein the nucleic acid has a nucleotide sequence of SEQ ID NO:1.

7. The isolated nucleic acid of claim 1, wherein the nucleic acid is amplified by primers that selectively hybridize under stringent ***hybridization conditions*** to the same sequence as primers selected from the group consisting of:

ATGCCGGGGGGCAAGAGAGGGCTG (SEQ ID NO:3);

CTGACCCTAAGCTCATAAGGATGAAC (SEQ ID NO:4);

CCACCTCATCATCCTGGATGACTTCC (SEQ ID NO:5);

TTAAAAGTGGATTTCATCTTTGTCAGATTCAGG (SEQ ID NO :6);

GGGGACCTCATTTACCATGCTGGAG (SEQ ID NO:7);

GATTCCCTCATCCACATTTTCAAAGGC (SEQ ID NO:8);

and wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

8. The isolated nucleic acid of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a homomeric channel.

9. The isolated nucleic acid of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a heteromeric channel.

10. An expression vector comprising the nucleic acid of claim 1.

11. A host cell transfected with the vector of claim 10.

12. A method of detecting a nucleic acid, the method comprising contacting a sample comprising a first nucleic acid with an isolated second nucleic acid of claim 1 and detecting hybridization of the second nucleic acid to the first nucleic acid, thereby detecting the first nucleic acid.

Description

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

FIELD OF THE INVENTION

The invention provides isolated nucleic acid and amino acid sequences of Eag2, antibodies to Eag2, methods of detecting Eag2, and methods of screening for modulators of Eag2 potassium channels using biologically active Eag2. The invention further provides, in a computer system, a method of screening for mutations of human Eag2 genes as well as a method for identifying a three-dimensional structure of Eag2 polypeptide monomers.

BACKGROUND OF THE INVENTION

Potassium channels are involved in a number of physiological processes, including regulation of heartbeat, dilation of arteries, release of insulin, excitability of nerve cells, and regulation of renal electrolyte transport. Potassium channels are thus found in a wide variety of animal cells such as nervous, muscular, glandular, immune, reproductive, and epithelial tissue. These channels allow the flow of potassium in and/or out of the cell under certain conditions. For example, the outward flow of potassium ions upon opening of these channels makes the interior of the cell more negative, counteracting depolarizing voltages applied to the cell. These channels are regulated, e.g., by calcium sensitivity, voltage-gating, second messengers, extracellular ligands, and ATP-sensitivity.

Potassium channels are made by alpha subunits that fall into 8 families, based on predicted structural and functional similarities (Wei et al., Neuropharmacology 35(7):805-829 (1997)). Three of these families (Kv, Eag-related, and KQT) share a common motif of six transmembrane domains and are primarily gated by voltage. Two other families, CNG and SK/IK, also contain this motif but are gated by cyclic nucleotides and calcium, respectively. The three other families of potassium channel alpha subunits have distinct patterns of transmembrane domains. Slo family potassium channels, or BK channels have seven transmembrane domains (Meera et al., Proc. Natl. Acad. Sci. U.S.A. 94(25):14066-71 (1997)) and are gated by both voltage and calcium or pH (Schreiber et al., J. Biol. Chem. 273:3509-16 (1998)). Another family, the inward rectifier potassium channels (Kir), belong to a structural family containing 2 transmembrane domains (see, e.g., Lagrutta et al., Jpn. Heart. J. 37:651-660 1996)), and an eighth functionally diverse family (TP, or "two-pore") contains 2 tandem repeats of this inward rectifier motif.

Potassium channels are typically formed by four alpha subunits, and can be homomeric (made of identical alpha subunits) or heteromeric (made of two or more distinct types of alpha subunits). In addition, potassium channels such as those composed of Kv, KQT and Slo or BK alpha subunits have often been found to contain additional, structurally distinct auxiliary, or beta, subunits. These beta subunits do not form potassium channels themselves, but instead they act as auxiliary subunits to modify the functional properties of channels formed by alpha subunits. For example, the Kv beta subunits are cytoplasmic and are known to increase the surface expression of Kv channels and/or modify inactivation kinetics of the channel (Heinemann et al., J. Physiol. 493:625-633 (1996); Shi et al., Neuron 16(4):843-852 (1996)). In another example, the KQT family beta subunit, minK, primarily changes activation kinetics (Sanguinetti et al., Nature 384:80-83 (1996)).

The Kv superfamily of voltage-gated potassium channels includes both heteromeric and homomeric channels that are typically composed of four subunits. Voltage-gated potassium channels have been found in a wide variety of tissues and cell types and are involved in processes such as neuronal integration, cardiac pacemaking, muscle contraction, hormone section, cell volume regulation, lymphocyte differentiation, and cell proliferation (see, e.g., Salinas et al., J. Biol. Chem. 39:24371-24379 (1997)).

A family of voltage-gated potassium genes, known as the "Eag" or ether a go-go family, was identified on the basis of a Drosophila behavioral mutation with a leg-shaking phenotype (see, e.g., Warmke & Ganetzky, Proc. Nat'l Acad. Sci. USA 91:3438-3442 (1994)). Family members from Drosophila and vertebrates have been cloned and fall into three subfamilies. One such subfamily is called the Eag subfamily and is represented, e.g., by Drosophila Eag (Warmke et al., Science 252:1560-1562 (1991); Bruggemann et al., Nature 365:445-447 (1993)), and rat, mouse, human, and bovine Eags (Ludwig et al., EMBO J. 13:4451-4458 (1994); Robertson et al. Neuropharmacology 35:841-850 (1996); Occhiodoro et al., FEBS Letters 434:177-182 (1998); Shi et al., J. Physiol. 115.3:675-682 (1998); Frings et al., J. Gen Physiol. 111:583-599 (1998)). A second subfamily, the Erg or "Eag-related gene" family is represented, e.g., by human erg (Shi et al., J. Neurosci. 17:9423-9432 (1997)). Finally, a third subfamily, the Elk or "Eag-like K.sup.+ gene" is represented, e.g., by Drosophila Elk (Warmke et al., Proc. Natl. Acad. Sci. 91:3438-3442 (1994)).

SUMMARY OF THE INVENTION

The present invention thus provides for the first time Eag2, a polypeptide monomer that is an alpha subunit of an voltage-gated potassium channel. Eag2 has not been previously cloned or identified, and the present invention provides the nucleotide and amino acid sequence of human Eag2.

In one aspect, the present invention provides an isolated nucleic acid encoding an alpha subunit of a potassium channel, wherein the subunit: (i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and (ii) comprises an amino acid sequence that has greater than about 70% identity to amino acids 720-988 of a human Eag2 amino acid sequence or comprises an amino acid sequence that has greater than about 85% identity to the amino acid sequence of SEQ ID NO:2.

In another aspect, the present invention provides an isolated nucleic acid that selectively hybridizes under moderately stringent hybridization conditions to a nucleotide sequence of SEQ ID NO:1.

In another aspect, the present invention provides an isolated nucleic acid that selectively hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1 or to a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:2.

In another aspect, the present invention provides a method of detecting a nucleic acid, by contacting the nucleic acid with a nucleic acid of the invention.

In another aspect, the present invention provides an isolated alpha subunit of a potassium channel, wherein the subunit: (i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; (ii) comprises an amino acid sequence that has greater than about 70% identity to amino acids 720-988 of a human Eag2 amino acid sequence or comprises an amino acid sequence that has greater than about 85% identity to the amino acid sequence of SEQ ID NO:2.

In one embodiment, the polypeptide specifically binds to polyclonal antibodies generated against SEQ ID NO:2.

In one embodiment, the nucleic acid encodes human Eag2. In another embodiment, the nucleic acid encodes SEQ ID NO:2. In another embodiment, the nucleic acid has the nucleotide sequence of SEQ ID NO:1. In another embodiment, the nucleic acid is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as primers selected from the group consisting of:

United States Patent **6,657,107**
Mahajan **December 2, 2003**

Polynucleotides encoding polypeptides having 8-oxoguanine DNA glycosylase activity and uses thereof

Abstract

The invention provides isolated mutM nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering maize mutM levels in plants in order to improve transformation efficiency, homologous recombination and/or targeted gene modifications. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions.

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Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. application Ser. No. 60/174,681 filed Jan. 6, 2000, which is herein incorporated in entirety by reference.

Claims

What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a nucleic acid sequence having at least 90% sequence identity over the entire length of SEQ ID NO: 1; wherein the percent identity is determined by the GAP algorithm under default parameters, wherein said sequence encodes a polypeptide having 8-oxoguanine DNA glycosylase (OGG) activity; and

(b) a nucleic acid sequence which is fully complementary to the nucleic acid sequence of (a).

2. A recombinant expression cassette comprising a member of claim 1 operably linked to a promoter.

3. A non-human host cell comprising the recombinant expression cassette of claim 2.

4. A transgenic plant comprising the recombinant expression cassette of claim 2.

5. The transgenic plant of claim 4, wherein said plant is a monocot.

6. The transgenic plant of claim 4, wherein said plant is a dicot.

7. The transgenic plant of claim 4, wherein said plant is selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.

8. A transgenic seed from the transgenic plant of claim 4.

9. A method of expressing 8-oxoguanine DNA glycosylase (OGG) in a plant, comprising:

(a) introducing into a plant cell a recombinant expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter;

(b) culturing the plant cell under plant cell growing conditions;

(c) regenerating a whole plant capable of expressing said polynucleotide; and

(d) expressing said polynucleotide for a time sufficient to modulate the level of mutM polypeptide in said plant.

10. The method of claim 9, wherein said plant is selected form the group consisting of: maize, soybean, safflower, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.

11. An isolated polynucleotide, which selectively hybridizes, under stringent ***hybridization conditions*** and a wash in 0.1.times.SSC at 60.degree. C., to the full-length complement of SEQ ID NO: 1, wherein stringent ***hybridization conditions*** comprise hybridization in 50% formamide, 1M NaCl, and 1% SDS at 37.degree. C., wherein the polynucleotidc, encodes a polypeptide having OGG activity.

12. The polynucleotide of claim 1, wherein the nucleic acid sequence of (a) has at least 95% sequence identity over the entire length of SEQ ID NO: 1.

13. The polynucleotide of claim 1, wherein the polynucleotide is SEQ ID NO: 1.

14. An isolated polynucleotide encoding the polypeptide of SEQ ID NO: 2.

Description

TECHNICAL FIELD

The present invention relates generally to plant molecular biology. More specifically, it relates to nucleic acids and methods for modulating their expression in plants.

BACKGROUND OF THE INVENTION

A variety of environmental agents such as gamma radiation, UV light in the 320-380 nm range, ozone, heat, and various chemicals cause oxidative damage to cellular DNA. Similarly, reactive oxygen species, hydroxyl radicals and superoxide and nitric oxide species generated in vivo cause oxidative damage to DNA (Friedberg, E. et al., in DNA repair and Mutagenesis, American Society of Microbiology Press, Washington D.C., pages 14-19, 1995). The precise nature of DNA modification varies depending upon the exposure and type of causative reagent. Such modifications as breakage of the phosphodiester bond have been reported, as well as oxidative stress induced illegitimate recombination in bacteria (Ouchane S. et al., EMBO J. 16: 4777-4787,1997). However, the most common result of oxidative damage is the oxidation of bases and sugars. Formamidopyrimidine (Fapy), 8-hydroxyguanine and 8-oxo-7,8 dihydrodeoxyguanosine are the most commonly observed base modifications following oxidative damage. Of these, 8-hydroxyguanine is considered highly mutagenic. It causes G:C to A:T transversions because 8-hydroxyguanine can pair with adenine and cytosine nucleotides with almost equal efficiencies during DNA replication (Shibutani A. et al., Nature 349: 431434, 1991; Maki, H. and Sekiguchi M., Nature 355: 273-275,1992).

Consequently, all living organisms have developed specific enzymatic pathways to remove such lesions and to maintain genomic stability. These enzymatic pathways have been very well characterized in bacteria and lower eukaryotes such as yeast. Implications of the involvement of oxidative DNA damage in the development of malignancies have also prompted a detailed analysis of these pathways in mammalian systems such as humans. These pathways have not been well studied however, in plants such as maize.

In E. coli, three genes labeled mutM, mutY, and mutT encode the enzymes responsible for the removal of Fapy and 8-hydroxyguanine lesions. Their gene products are members of the DNA glycosylase

family. The mutY gene product specifically removes the unmodified A from the 8-hydroxyguanosine: A pair. The mutT gene product, on the other hand, preferentially hydrolyzes 8-oxo-7,8 dihydrodeoxyguanosine thereby preventing its incorporation in DNA. E. coli mutants of these genes show a mutator phenotype with a 10-1000 fold increase in transversions compared to wild type. In addition to the mutator phenotype, E.coli mutM mutants show increased illegitimate or non-homologous recombination. Furthermore, the mutM gene product suppresses this illegitimate recombination (Onda, M. et al., Genetics 151:439446, 1999). Thus, overexpression of the mutM gene product may be used as a tool to suppress mutations in general and oxidative stress induced non-homologous recombination in particular.

Recent studies have revealed the presence of mutM orthologues in yeast, human, and Arabidopsis thaliana (van der Kemp PA et al., PNAS 93:5197-5202, 1996; Arai, K. et al., Oncogene 14:2857-2861,1997; Radicella, JP et al., PNAS 94:8010-8015,1997; Ohtsubo, T. et al., Mol. Gen. Genet. 259:577-590,1998). The present invention presents a full-length cDNA encoding a maize orthologue of E. coli mutM. Unlike the animal mutM orthologues, the maize enzyme contains a C-terminal region of alternating acidic and basic amino acid residues and a putative nuclear localization signal as shown in Example 4. The mutM orthologue of the present invention may be useful as a suppresser of DNA mutations which are induced by oxidative damage. Furthermore, it may be used to reduce illegitimate recombination thereby increasing frequencies of homologous recombination and transformation. Control of these processes has important implications in the creation of novel recombinantly engineered crops such as maize. The present invention provides for these and other advantages.

SUMMARY OF THE INVENTION

Generally, it is the object of the present invention to provide nucleic acids and proteins relating to maize mutM. It is an object of the present invention to provide expression cassettes, host cells and transgenic plants comprising the nucleic acids of the present invention, and methods for modulating, in a transgenic plant, the expression of the nucleic acids of the present invention in order to improve the efficiency of homologous recombination, transformation efficiency or to induce targeted gene changes. It is also an object of the present invention to provide antibody compositions for detecting the polypeptides of the present invention.

In other aspects the present invention relates to: 1) recombinant expression cassettes, comprising a nucleic acid of the present invention operably linked to a promoter, 2) a non-human host cell into which has been introduced the recombinant expression cassette, and 3) a transgenic plant comprising the recombinant expression cassette.

Definitions

Units, prefixes, and symbols may be denoted in their Si accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges recited within the specification are inclusive of the numbers defining the range and include each integer within the defined range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. Unless otherwise provided for, software, electrical, and electronics terms as used herein are as defined in The New IEEE Standard Dictionary of Electrical and Electronics Terms (5.sup.th edition, 1993). The terms defined below are more fully defined by reference to the specification as a whole.

By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple

United States Patent **6,635,446**
Ramesh , et al. **October 21, 2003**

WIP, a WASP-associated protein

Abstract

Described herein is a novel gene and its product, WIP, which associates with WASP. The subject invention relates to the isolated WIP gene or cDNA (see FIGS. 1A-1B); nucleic acid probes, which can be fragments of the WIP gene or WIP cDNA or full-length; nucleic acid primers, which are fragments of WIP cDNA or the WIP gene; methods of assessing cells (e.g., for diagnostic purposes) for the presence of WIP DNA, (e.g., wildtype or mutated) or for the absence or occurrence of a reduced level of WIP DNA; WIP mRNA; WIP or WIP fragments, such as those which are useful to generate antibodies which bind WIP; and antibodies which bind WIP. Also the subject of this invention are methods of treating conditions in which WIP and/or WASP DNA or protein is deficient (in quantity) and/or defective (e.g., mutated/altered) such that an individual is adversely affected (e.g., has Wiskott-Aldrich Syndrome); methods of altering or regulating WASP and its functions; and methods of altering actin content, actin polymerization or both in cells, such as human lymphoid cells (e.g., .beta. lymphocytes). A further subject of this invention is an assay to identify drugs which alter (e.g., enhance) the activity of WIP or expression of WIP DNA.

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Goverment Interests

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by grants AI37130 and AI35714 from National Institutes of Health. The Government has certain rights in the invention.

Parent Case Text

RELATED APPLICATION(S)

This application is a continuation application of PCT/US98/27501, entitled "WIP, A WASP-ASSOCIATED PROTEIN", filed Dec. 22, 1998, which claims the benefit of U.S. Provisional Application No. 60/101,457 filed Sep. 23, 1998, entitled "WIP, A WASP-ASSOCIATED PROTEIN, INDUCES ACTIN POLYMERIZATION AND REDISTRIBUTION IN LYMPHOID CELLS", and U.S. Provisional Application No. 60/068,533 filed Dec. 23, 1997, entitled "WIP, A WASP-ASSOCIATED PROTEIN, INDUCES ACTIN POLYMERIZATION AND REDISTRIBUTION IN LYMPHOID CELLS". The entire teachings of each application are incorporated herein by reference.

Claims

What is claimed is:

1. An isolated nucleic acid which encodes WIP, a WASP-interacting protein comprising SEQ ID NO:1.

2. The nucleic acid of claim 1 wherein the nucleic acid encodes an amino acid sequence comprising SEQ ID NO:2.

3. An isolated fragment of SEQ ID NO:1 which encodes WIP, a WASP-interacting protein, wherein the fragment includes at least 10 consecutive nucleotides in the coding region of SEQ ID NO: 1 which are 5' of nucleotide 380 of SEQ ID NO: 1.

4. An isolated nucleic acid which encodes WIP, a WASP-interacting protein, and which selectively hybridizes under moderately stringent hybridization conditions to the complement strand of SEQ ID NO: 1, said moderately stringent ***hybridization conditions*** comprising washing with 0.5.times.SSC containing 0.1% SDS at 65.degree. C. for 1 hour, and wherein said isolated nucleic acid comprises at least 10 consecutive nucleotides of the coding region of SEQ ID NO: 1 which are 5' of nucleotide 380 of SEQ ID NO: 1.

5. A recombinant nucleic acid construct comprising the nucleic acid of claim 1.

6. The recombinant nucleic acid construct of claim 5 wherein the nucleic acid encodes an amino acid sequence comprising SEQ ID NO: 2.

7. The recombinant nucleic acid construct of claim 5 wherein the nucleic acid is operably linked to an expression control sequence.

8. A host cell comprising the recombinant nucleic acid construct of claim 7.

9. The host cell of claim 8 wherein the nucleic acid is operably linked to an expression control sequence, whereby WIP is expressed when the host cell is maintained under conditions suitable for expression.

10. A method for producing a WASP-interacting protein comprising:

a) introducing into a host cell a nucleic acid construct comprising a nucleic acid comprising full length SEQ ID NO:1; and

b) maintaining the host cell produced in step a) under conditions whereby the nucleic acid is expressed and the WASP-interacting protein is produced.

11. A isolated nucleic acid which encodes an amino acid sequence comprising SEQ ID NO:2.

12. A recombinant nucleic acid construct comprising the nucleic acid of claim 11.

13. The recombinant nucleic acid construct of claim 12, wherein the nucleic acid is operably linked to an expression control sequence.

14. A host cell comprising the recombinant nucleic acid construct of claim 13.

15. A method for producing a WIP polypeptide comprising culturing the host cell of claim 14 under conditions suitable for expression of the recombinant nucleic acid construct.

16. An isolated nucleic acid comprising of SEQ ID NO:1.

17. An isolated nucleic acid construct comprising the nucleic acid of claim 16.

18. The recombinant nucleic acid construct of claim 17, wherein the nucleic acid is operably linked to an expression control sequence.

19. A host cell comprising the nucleic acid construct of claim 18.

20. A method of producing a WIP polypeptide comprising culturing the host cell of claim 19 under conditions suitable for expression of the nucleic acid construct.

21. An isolated nucleic acid which encodes SEQ ID NO:2.

22. An isolated nucleic acid fragment of SEQ ID NO: 1, wherein the fragment includes at least 10 consecutive nucleotides in the coding region of SEQ ID: 1 which are 5' of nucleotide 380 of SEQ ID: 1.

23. An isolated fragment of SEQ ID NO: 1 which encodes WIP, a WASP-interacting protein, and which selectively hybridizes under moderately stringent ***hybridization conditions*** to the complement strand of SEQ ID NO: 1, said moderately stringent ***hybridization conditions*** comprising washing with 0.5.times.SSC containing 0.1% SDS at 65.degree. C. for 1 hour, and wherein said isolated fragment comprises at least 10 consecutive nucleotides of the coding region of SEQ ID NO: 1 which are 5' of